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Abstract: Distal renal tubular acidosis (dRTA) is a tubular disorder with a primary defect of urinary acidification and acid excretion in the collecting duct system. Consequently, patients develop hyperchloremic metabolic acidosis with an inappropriately alkaline urine. Inherited forms of dRTA are due to mutations in at least three distinct genes: SLC4A1, ATP6V1B1, ATP6V0A4. Mutations in SLC4A1-(AE1) are inherited either in an autosomal dominant manner or in a recessive one. ATP6V1B and ATP6V0A4 mutations affect two different subunits of the vacuolar H+-ATPase proton-pump, the B1 and a4 subunits, and are inherited in an autosomal recessive manner. Clinical manifestations of inherited forms of dRTA usually occur during infancy or childhood. However, heterozygous carriers of ATP6V1B1 and ATP6V0A4 mutations may have a higher risk of developing nephrolithiasis and nephrocalcinosis in adulthood, respectively. In full forms of dRTA, patients may present with mild clinical symptoms, such as mild metabolic acidosis and incidental detection of kidney stones, as well as with more severe manifestations such as failure to thrive, severe metabolic acidosis, and nephrocalcinosis. Progressive sensorineural hearing loss develops in the majority of patients with recessive dRTA (ATP6V1B1 and ATP6V0A4 mutations). Some patients with recessive dRTA may also develop abnormal widening of the vestibular aqueduct. This review will discuss our current understanding of the pathophysiology of inherited forms of dRTA, diagnosis and prognosis of patients, and therapy.

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The authors declare that they have no conflict of interest.

ABSTRACT

Distal renal tubular acidosis (dRTA) is a tubular disorder with a primary defect of urinary acidification and acid excretion in the collecting duct system. Consequently, patients develop hyperchloremic metabolic acidosis with an inappropriately alkaline urine. Inherited forms of dRTA are due to mutations in at least three distinct genes: *SLC4A1*, *ATP6V1B1*, *ATP6V0A4.* Mutations in *SLC4A1*-(AE1) are inherited either in an autosomal dominant manner or in a recessive one. *ATP6V1B* and *ATP6V0A4* mutations affect two different subunits of the vacuolar H^+-ATPase proton-pump, the B1 and a4 subunits, and are inherited in an autosomal recessive manner. Clinical manifestations of inherited forms of dRTA usually occur during infancy or childhood. However, heterozygous carriers of *ATP6V1B1* and *ATP6V0A4* mutations may have a higher risk of developing nephrolithiasis and nephrocalcinosis in adulthood, respectively. In full forms of dRTA, patients may present with mild clinical symptoms, such as mild metabolic acidosis and incidental detection of kidney stones as well as with more severe manifestations such as failure to thrive, severe metabolic acidosis, and nephrocalcinosis. Progressive sensorineural hearing loss develops in the majority of patients with recessive dRTA (*ATP6V1B1* and *ATP6V0A4* mutations). Some patients with recessive dRTA may also develop abnormal widening of the vestibular aqueduct. This review will discuss our current understanding of the pathophysiology of inherited forms of dRTA, diagnosis and prognosis of patients, and therapy.

RENAL ACID EXCRETION

Next to the ventilation of CO_2 by the lungs, the kidneys play a central role in the long-term control of acid-base homeostasis. The daily excretion of acid and the regeneration of approximately 1 mmol bicarbonate per kg bodyweight (e.g. 70 mmoles in an average person of 70 kg body weight per day) are critical tasks. The importance of these processes becomes most evident in syndromes or diseases affecting overall kidney function or more specifically in forms of acquired or inherited renal tubular acidosis.

Maintenance and control of systemic acid-base balance by the kidney is achieved through three major processes: 1) the reabsorption of filtered bicarbonate, 2) the excretion of acid mostly in the form of ammonium and titratable acidity, and 3) by the *de novo* synthesis of bicarbonate to replenish bicarbonate lost in metabolism [1].

The kidneys filter daily about 180 litres of primary urine containing a total of approximately 4500 mEq bicarbonate which in a healthy person is entirely reabsorbed along the nephron. About 80% of the filtered bicarbonate are reclaimed in the proximal tubule via secretion of protons by NHE sodium-proton exchangers (mostly the NHE3/*SLC9A3* isoform) and proton pumps (H^+-ATPases) and, as suggested recently the sodium-dependent bicarbonate cotransporter (NBCn2) [2]. Because of the luminal activity of carbonic anhydrases (Carbonic anhydrase type IV (CAIV)) the formation of CO_2 and H_2O from HCO_3^- and H^+ is facilitated. CO_2 and H_2O then diffuse into proximal tubule cells where the process is reversed by the cytosolic carbonic anhydrase type II (CAII). The resulting HCO_3^- is released into blood by the basolateral sodium-bicarbonate cotransporter (NBCe1/*SLC4A4*) whereas protons are recycled into urine across the luminal membrane (**Figure 1**). A fraction of bicarbonate is also reabsorbed through the paracellular pathway in the proximal tubule driven by the luminal accumulation of chloride and the lumen-negative potential.

The remaining bicarbonate (approx. 20 % of the filtered load) is then reabsorbed along the thick ascending limb of the loop of Henle by transcellular mechanisms similar to the proximal tubule.

Metabolism consumes bicarbonate (i.e. in the urea cycle) and produces acids that require buffering by bicarbonate. The kidney replenishes bicarbonate by *de novo* generation of bicarbonate from ammoniagenesis in the proximal tubule and by

hydration of CO_2 in acid-secretory type A intercalated cells. In the proximal tubule, glutamine is taken up mostly from blood and fueled into ammoniagenesis and gluconeogenesis releasing ammonia and bicarbonate ions. Ammoniagenesis is stimulated during acidosis (by enhanced glutamine uptake and higher enzymatic fluxes) and contributes to the renal adaption. Renal ammonium excretion is a process involving several steps. First ammonium is secreted into urine in the proximal tubule (a fraction is also released back into circulation), mostly reabsorbed by the Na/K/2Cl-cotransporter NKCC2 in the thick ascending limb of the loop of Henle, accumulated in the interstitium and finally secreted by the cells lining the collecting duct system into urine in the form of ammonia (see below).

Final urinary acidification and fine-tuning of renal acid-excretion occurs in the collecting system consisting of the connecting tubule, the cortical and medullary parts of the collecting duct [3]. The first intercalated cells appear already in the late distal convoluted tubule.

Acid-secretory type A intercalated cells not only mediate ammonia excretion into urine but are also responsible for urinary acidification coupled to *de novo* synthesis of bicarbonate (**Figure 2**). CO_2 is hydrated with the help of the cytosolic CAII forming protons and bicarbonate. Bicarbonate is released into blood via the basolateral chloride-bicarbonate exchanger AE1 (Anion exchanger 1, SLC4A1) whereas protons are pumped into urine by vacuolar-type H^+-ATPases located in the luminal membrane [3-4]. As discussed below, rare genetic mutations in SLC4A1 or two different subunits of the multimeric H^+-ATPase (consisting of more than 14 subunits with multiple isoforms) cause inherited forms of distal renal tubular acidosis (dRTA) [5-7]; Secretion of protons into urine acidifies urine to a maximal pH of around 4.5 - 4.0. Further acidification of urine is impossible as proton pumps must work against a steep proton gradient (intracellular pH 7.2, luminal pH 4.5). However, one liter of urine of pH 4.5 contains only 30 μmoles protons, a minute amount compared to the requirement to excrete 70 mmoles of acid. Urinary buffers, so-called titratable acidity (the term refers to the method to measure titratable acidity by back-titrating acidified urine), help to buffer protons and thereby to increase the amount of excreted acid. The main “titratable acid» is phosphate, creatinine and urate also contribute to variable extents. Proton secretion is also tightly coupled to ammonia secretion where

luminal ammonia (NH_3) captures free protons and is trapped in urine in the form of ammonium (NH_4^+). Ammonia secretion by intercalated cells (and also by neighboring principal cells) is mediated by two related gas channels belonging to the family of the rhesus blood group proteins, namely RhBG and RhCG (**Figure 2**) [8-10].

The sum of urinary ammonium plus titratable acidity minus bicarbonate is called net acid secretion. For the sake of simplicity, urinary phosphate can be taken as approximation for titratable acidity and urinary bicarbonate can be neglected when urine pH is below pH 6.5 [8].

The activity of type A intercalated cells and hence net acid secretion is enhanced during acidosis and decreased during alkalosis.

Next to type A intercalated cells, a second type of intercalated cells, type B intercalated cells, is expressed in the distal convoluted tubule, connecting tubule and cortical collecting duct. These cells harbor the chloride/bicarbonate exchanger pendrin (SLC26A4) on their luminal membrane and play an important role in the secretion of bicarbonate during alkalosis and the reabsorption of chloride [11-12]. The latter may be important for the control of NaCl homeostasis and blood pressure control [13-18].

CASE REPORT

A 35-year old male patient with recurrent urolithiasis and nephrocalcinosis was referred to our stone clinic for metabolic evaluation. He was diagnosed with distal renal tubular acidosis when he presented at the age of 6 years with severe metabolic acidosis (pH 6.98, bicarbonate 3.3 mmol/l) and alkaline urine pH of 7.0. Ultrasound of the kidneys demonstrated bilateral medullary nephrocalcinosis. After careful analysis of his pedigree an autosomal recessive inheritance was suspected. Consequently, alkali treatment with potassium citrate and sodium bicarbonate was initiated. However, due to non-adherence he suffered from repetitive episodes of nephrolithiasis during adolescence and young adulthood. Stone analysis revealed 100% calcium phosphate. Additionally, as a consequence of the repeated pain therapy with opioids he became opioid dependent. At presentation in our stone clinic he had developed chronic kidney disease KDIGO stage G 3a-b and sensorineural hearing loss. Ultrasound of his kidneys demonstrated bilateral nephrocalcinosis (**Figure 3**). Adherence to therapy was still problematic since hypokalemia (serum

potassium 3.1 mmol/l) and metabolic acidosis (bicarbonate 15 mmol/l) were still present. Bone densitometry indicated osteopenia with normal levels of calcium, parathyroid hormone, 25-OH- and 1,25-$(OH)_2$-Vitamin D_3. Serum phosphate was low (0.67 mmol/l). Genetic analysis in the Department of Genetics at the European Georges Pompidou Hospital in Paris revealed a homozygous mutation (p.Gln753*) in the *ATP6V0A4* gene encoding for the a4 subunit of the vacuolar H^+-ATPase. Despite ongoing repetitive kidney stone episodes, his kidney function remained stable over the last 3 years.

MECHANISMS OF INHERITED FORMS OF DISTAL RENAL TUBULAR ACIDOSIS

Inherited forms of renal acid-base disturbances are rare and caused by mutations in transport proteins and enzymes located in acid-secretory intercalated cells in the collecting duct system, mutations of components of the angiotensin II - aldosterone system regulating renal acid excretion, or by mutations leading to malformations of the kidney [1]. The various types of renal tubular acidosis affect mostly specific transport pathways localized in distinct nephron segments which provide the basis for the nomenclature of these acid-base disturbances. In the following we will focus on defects underlying type I renal tubular acidosis (RTA I, classic type) or distal renal tubular acidosis (dRTA).

Classic dRTA is characterized by the inability to acidify urine below pH 5.3 in the presence of metabolic acidosis. Consecutively, the excretion of ammonium and titratable acids is also reduced leading to an overall reduction in urinary acid excretion [1]. Patients develop hyperchloremic metabolic acidosis usually with a normal anion gap often associated with hypokalemia. During childhood and adolescence, failure to thrive, growth retardation, rickets, and nephrolithiasis or nephrocalcinosis may occur and lead to the initial diagnosis. Patients may also develop polyuria which may be triggered by the reduced capacity to concentrate urine due to hypercalciuria, hypokalemia or nephrocalcinosis [19-21].

Incomplete dRTA presents also with inadequate urinary acidification but patients usually have normal blood gases, i.e. normal blood pH and bicarbonate. The

defect can be revealed with the various types of acid challenge tests (ammonium chloride or fludrocortisone-furosemide test, see below) where urine pH does not acidify below 5.3 [22].

To date, mutations in genes encoding for three distinct transport proteins have been identified to cause classic dRTA: in the chloride-bicarbonate exchanger AE1/*SLC4A1* or in the B1/*ATP6V1B1 and* a4/*ATP6V0A4* subunits of the vacuolar-type H^+-ATPase [5-6,23-24]. However, not all cases of inborn dRTA can be explained by mutations in these genes suggesting that mutations in additional genes may contribute to inherited dRTA. Candidate genes may include the K^+/Cl^--cotransporter KCC4 (SLC12A7) [25], the Forkhead transcription factor Foxi1 [26], the Cl^-/HCO_3^- -exchanger SLC26A7 [27], the ammonia channel RhCG (SLC42A3) [8], the hensin (DMBT1)-CXCL12 signal complex [28-29], or other H^+-ATPase subunits [30]. In Europe, mutations in *ATP6V1B1* and *ATP6V0A4* appear to be more prevalent whereas in other regions, the relative occurrence of mutations may be different.

Mutations in *SLC4A1* can be inherited in an autosomal dominant manner (heterozygous mutations) but also with an autosomal recessive inheritance (homozygous mutations). In contrast, mutations in the *ATP6V1B1* and *ATP6V0A4* genes follow an autosomal recessive pattern but the significance of heterozygous mutations (i.e. only one mutated allele detectable) has recently been discussed (see below)[31].

The proton pump consists of a protein complex of two major domains, the cytosolic catalytic V_1 domain hydrolyzing ATP (with 8 subunits A-H) and the membane-bound V_0-Domäne mediating the proton transfer with the a, c, c“, d, and e subunits [32]. The B1 subunit is part of the V1-domain whereas the a4 subunit belongs to the V0-domain (**Figure 2**). The B1 subunit is found only in a few organs including kidney, inner ear, epididymis and lung. In kidney, the B1 subunit is highly enriched in all types of intercalated cells but is also detected at lower levels in the thick ascending limb of Henle. The a4 subunit is also enriched in all types of intercalated cells but is also highly abundant in the proximal tubule and in the thick ascending limb of the loop of Henle [33]. The subunit is also expressed in epididymis and the stria vascularis of the inner ear [24,34]. The expression of both subunits, B1

and a4, in the inner ear may explain the occurrence of sensorineural deafness in patients with mutations in these subunits. Nevertheless, the progression of sensorineural deafness is variable in patients and does not respond to alkali therapy [35-36]. Some patients may also develop dizziness possibly because of an enlarged vestibular aqueduct (EVA) observed in some but not all patients [36]. Whether alterations in the function of proton pumps in the epididymis occur and affect male fertility in these patients has remained unknown.

Based on experiments in yeast and cell culture models it appears that most of the mutations identified in the B1 subunit cause either dysfunction or impaired assembly of the protein complex [37-38]. Accordingly, mice lacking the B1 subunit have a reduced capacity to acidify urine and develop more severe metabolic acidosis when acid-loaded. When crossed with hypercalciuric mice, B1 deficient mice develop severe nephrocalcinosis with hydronephrosis [39,19,40].

Lack of the a4 subunit in mice causes severe dRTA with hypokalemia, nephrocalcinosis, and reduced bone mineral density [41-42]. The mice develop also a massive hearing loss and a reduced sense of smell. The absence of the a4 subunit from the proximal tubule is associated with low molecular weight proteinuria suggesting an important role of this subunit in receptor-mediated endocytosis [42]. In at least one series of patients with mutations in either *ATP6V1B1* or *ATP6V0A4*, mutations in the latter were associated with a more severe clinical presentation and reduced kidney function [42].

The chloride-bicarbonate exchanger AE1 (SLC4A1) is expressed both in acid-secretory type A intercalated cells and red blood cells. Mutations in SLC4A1 cause either dRTA or red blood cell abnormalities including spherocytosis or South-East Asian ovalocytosis (SAO). Importantly, most mutations cause either dRTA or hematological abnormalities but only few mutations affect both systems. The mode of inheritance is usually autosomal dominant but few autosomal recessive mutations have been described. The most frequent recessive mutation, G701D, causes dRTA that can be associated with hemolytic anemia. Interactions of AE1 with the chaperone glycophorin have been identified to underlie the separation of renal and red blood cell mutations as this molecule is only expressed in red blood cells and is able to rescue "renal" mutations bringing them to the red blood cell membrane [43]. A

series of additional mutations has been identified that are more common in South-East Asia and are mostly associated with a red blood cell phenotype. It has been speculated that some of these mutations may confer resistance to malaria [44]. In contrast to the recessive mutations, patients with a Caucasian background harbor more frequently dominant mutations, the R589H being the most common one, that rather causes dRTA [45-46]. Several types of AE1 mutations have been described that may cause either intracellular retention of mutant proteins or even mistargeting to the luminal membrane of type A intercalated cell models [47-49]. In mice, complete absence of AE1 causes severe metabolic acidosis and reduced renal excretion [50]. Introduction of the R589H mutation in mice (in mice this mutation corresponds to R607H) causes dysfunction of intercalated cells with reduced expression of proton pumps [45].

INHERITED DISTAL RENAL TUBULAR ACIDOSIS AS AN UNDERLYING CAUSE OF NEPHROCALCINOSIS OR KIDNEY STONES IN ADULTS

Nephrocalcinosis is caused by various disorders with different pathophysiologies including e.g. primary hyperoxalurias, sarcoidosis, medullary sponge kidney, primary hyperparathyroidism, distal RTA and others. Depending on the underlying cause patients may develop chronic kidney disease (CKD) with progression to end stage kidney disease requiring renal replacement therapy. Thus, correct and timely diagnosis is of prime importance. Clinical manifestation of inherited dRTA can vary among patients depending on the underlying gene mutation. Hereditary recessive distal RTA due to B1 or a4 subunit mutations of the H^+-ATPase typically manifests during infancy or childhood and presents with severe symptoms such as vomiting, failure to thrive, diarrhea or constipation, polyuria, nephrocalcinosis or rickets/osteomalacia. However, few cases may present with milder symptoms including a mild metabolic acidosis, hypocitraturia, incidental detection of kidney stones or renal calcification. Particularly, patients with autosomal dominant distal RTA due to mutations in the *SLC4A1* gene may present first clinical symptoms only during adulthood.

As a consequence of metabolic acidosis skeletal buffers such as carbonate and phosphate in combination with calcium are removed from the bones resulting in bone demineralization and subsequently in hypercalciuria. Additionally, expression of renal calcium transport proteins is decreased in metabolic acidosis further promoting

calcium excretion and thus development of nephrocalcinosis and kidney stones (**Figure 3**) [19,51].

Consequently, in patients with nephrocalcinosis or repetitive episodes of kidney stones distal RTA is an important differential diagnosis and should be considered, particularly if stone analysis detects calcium phosphate containing stones in presence of metabolic acidosis or if there is evidence of impaired hearing or deafness.

DIAGNOSIS OF INHERITED dRTA

Distal RTA results from a defective urinary acidification and is characterized by an inappropriate alkaline urine pH in the context of a hyperchloremic, normal anion gap metabolic acidosis with preserved GFR. The mutated genes, namely *B1 (ATP6V1B1)* and *a4 (ATP6V0A4)* subunit as well as *AE1 (SLC4A1)*, are also expressed in extrarenal tissues, including the epididymis and cells of the stria vascularis of the inner ear (B1 and a4), and erythrocytes (AE1), respectively. The diagnosis is primarily based on typical clinical and laboratory abnormalities and confirmed by genetic analysis. The phenotype includes renal and extrarenal clinical symptoms. Specialized tests to test for urinary acidification capacity are mentioned below and are mainly required for diagnosis of incomplete forms of dRTA.

Short ammonium chloride loading test

Diagnosis of the renal defect is established by the short ammonium chloride loading test (= the short test of urinary acidification) that has been refinded and validated by Wrong and Davies in a seminal study several decades ago [22]. The principle of the short ammonium chloride loading test is based on the principal mechanism of hydrogen ion or acid secretion by the « healthy» kidney, namely excretion of all hydrogen ions combined with ammonia (NH_3) as ammonium (NH_4^+). Meanwhile, several animal studies have confirmed the crucial role of ammoniagenesis and ammonium excretion in renal acid excretion [52-55].

The original protocol of the test is explained briefly: After emptying the bladder, urine is collected hourly under paraffin oil and thymol or toluene for 10 hours. After two hourly collections of urine, ammonium chloride capsules are given orally at a dose of

0.1 g (≈ 1.9 meq) per kg body weight over an hour. Blood gas analysis is performed before and after two to four hours after ingestion of ammonium chloride. Urinary pH is measured hourly using a pH electrode. In this test, urinary pH below 5.3 excludes an urinary acidification defect and the test is terminated. Wrong and Davies had investigated a total of 68 subjects, 10 healthy controls and 58 patients with different forms of renal diseases, including general renal failure without evidence of tubular abnormality, complete or incomplete renal tubular acidosis, and prolonged hypercalcemia and others. By using the ammonium chloride test, the authors demonstrated that the test is a reliable method to evaluate the ability of the kidney to excrete acid. Recently the test has been applied in several human studies [56-58]. Mostly, it has been used to selectively screen for complete or incomplete forms of distal renal tubular acidosis in recurrent kidney stone formers [57-58]. However, although the ammonium chloride loading test is still the «gold standard» to test for defective urinary acidification, many patients suffer from unpleasant gastrointestinal side effects of ammonium chloride such as nausea and vomiting and also are not pleased about the duration of the test for a maximum of 8 hours. Thus, Walsh et al. developed a more simple but effective, and well-tolerated alternative test that will be discussed in detail in the next paragraph.

The simultaneous furosemide and fludrocortisone test as an alternative to ammonium chloride

The simultaneous furosemide plus fludrocortisone test (f+f test) is based on previous studies describing a stimulation of H^+ secretion in response to oral furosemide application [59]. The f+f test has been tested in complete and incomplete dRTA and it is less specific than the gold standard ammonium chloride test. The test is thought to be based on the stimulation of electrogenic sodium reabsorption by the epithelial sodium channel ENaC in the collecting duct system due to enhanced delivery of sodium after blockade of Na^+-reabsorption by the loop diuretic furosemide in the TAL [60]. Higher activity of ENaC would cause a more lumen-negative potential in the collecting duct system and thereby increase the driving force for proton secretion. The mineralocorticoid fludrocortisone would stimulate ENaC activity but also direct effects of aldosterone on H^+-ATPase activity have been described [61-62]. More recently, an alternative explanation has been provided whereby furosemide would stimulate NHE3-dependent proton secretion in the TAL and thereby increase

urinary acidification [63]. Why TAL proton secretion would be reduced in dRTA patients is unclear but could be related to the expression of the ATP6V1B1 and ATP6V0A4 transcripts in the TAL [33]. However, a more recent study conducted in healthy human volunteers provides support for the initial hypothesis that furosemide –induced urinary acidification requires ENaC-activity as the furosemide-induced drop in urinary pH was blunted when the ENaC-inhibitor amiloride was coadministered [64].

In a first study with the f+f test, Walsh et al. investigated 8 patients with previously diagnosed dRTA and a control group of 11 healthy probands [56]. All participants were subjected to a short ammonium chloride test followed by the f+f test. Briefly, a baseline urine sample was collected from all participants followed by oral administration of 40mg furosemide and 1mg fludrocortisone. Urine collection was performed hourly and urine pH was measured using an electrode pH meter for 6 hours after the baseline sample. Notably, there were no adverse effects with the f+f test. All healthy probands were able to acidify their urine below pH 5.3 with the f+f test or the ammonium chloride test while urine pH of dRTA patients remained above pH 5.3 indicating defective urinary acidification. In a follow-up study the f+f test was further used in a preselected cohort of kidney stone formers [57]. In this study the authors confirmed a distinct sensitivity and excellent negative predictive value of this test to exclude incomplete dRTA in patients with kidney stones or nephrocalcinosis or both. However, this study was retrospectively performed and only patients with a clinical suspicion for an acidification defect were tested. Consequently, the reliability of the f+f test, especially in the diagnosis of incomplete dRTA, remains to be further validated by ideally a prospective blinded study in a cohort of unselected patients. Additionally due to limited specificity patients tested negative with f+f test may require confirmation by the ammonium chloride loading test. This finding has also been confirmed in another study by Dhayat and colleagues who prospectively subjected an unselected cohort of 170 stone formers to sequential ammonium chloride and f+f testing [65]. Furthermore, the authors also tested for non-provocative laboratory parameters to predict incomplete dRTA and could demonstrate by using a morning fasting urinary pH at a threshold of > 5.3 with a plasma potassium threshold of > 3.8 mmol/l that incomplete dRTA can reliably be excluded. Thus, future studies are required to verify the value and impact of the f+f test in diagnosing incomplete dRTA.

Hearing test

The ATP6V1B1 and ATP6V0A4 are also expressed in extrarenal tissues such as in the stria vascularis of the inner ear. Thus, the majority of patients with the recessive forms of dRTA develop progressive bilateral sensorineural hearing loss which is interestingly more common in patients with *B1* mutations than in subjects with *a4* mutations [46,35,6,41-42]. Some patients also present with other abnormalities of the auricular system such as abnormal widening of the vestibular aqueduct (enlarged vestibular aqueduct, EVA) which is usually bilaterally present (**Figure 4**) [36]. However, this type of abnormalities is not specific for hereditary dRTA since they may also be present in patients with Pendred or Branchio-oto-renal syndrome.

To test for sensorineural hearing abnormalities a standard audiogram has to be performed investigating masked and unmasked bone and air conduction at different frequencies. To detect other auricular abnormalities, both, MRI or CT can be used for the diagnosis of enlarged vestibular aqueducts (**Figure 4**) [36].

Patients with inherited dRTA due to mutations in *SLC4A1* may present concomitantly with Southeast Asian ovalocytosis (SAO), mainly in the Malay archipelago, the Philippines, Indonesia and southern Thailand. SAO is a hematologic disease that is clinically characterized by hemolytic anemia, oval shape erythrocytes in the peripheral blood smear, and the presence of the hemizygous deletion of amino acids 400-408 (also known as SAO mutation) [66].

LONG-TERM PERSPECTIVES

Clinical outcome/ Progression to CKD

To date, very few data exist on long-term clinical outcome of inherited dRTA patients. Most studies have primarily investigated the genotype-phenotype characteristics of these patients at diagnosis. The most recent report has investigated one of the largest cohorts of patients with dRTA so far [67]. Among 89 patients clinically diagnosed with inherited dRTA, mutations in *ATP6V1B1, ATP6V0A4, and*

SLC4A1 were found in 71.9% of all subjects. There was no significant difference regarding male and female distribution for all genes. Mean age of onset was around 5.5 years, however, patients with SLC4A1 mutations typically present at an older age (12-13 years-old) compared to subjects with *ATP6V1B1* and *ATP6V0A4* mutations. As expected, sensorineural hearing loss was present in the majority of cases with *ATP6V1B1* (92%) and *ATP6V0A4* (56.7%) mutations, with a significantly earlier onset in patients carrying the *ATP6V1B1* mutation. Another common finding was nephrocalcinosis that was detected in 93.6% of all mutated patients without differences among the different types of mutated genes. In this cohort, hypokalemia was more frequent and severe in patients with H^+-ATPase mutations compared to subjects with SLC4A1 mutations. Notably, a significant proportion of subjects with pathogenic mutations (31.3%) suffered from chronic kidney disease (CKD, defined according to the KDIGO criteria: eGFR < 90 ml/min/1.73m^2) during the long-term follow-up, presenting after pubertal growth spurt. These findings are novel and of particular importance since inherited dRTA was always considered as a “benign” disease with regard to kidney function [46]. However, the pathophysiology of CKD is unclear and has been discussed to be caused by tubulo-interstitial damage due to nephrocalcinosis and persistent hypokalemia. In addition, repeated pre-renal hits with acute kidney injury may also result in chronic kidney damage. Further studies are required to confirm these findings in larger cohorts. A previous smaller study including 19 children with genetically confirmed inherited dRTA also reported earlier age of onset in patients with *ATP6V1B1* and *ATP6V0A4* mutations compared to subjects with *SLC4A1* mutations [46]. Metabolic acidosis was more profound in children with *ATP6V0A4* mutations. Interestingly, in this cohort a substantial number of patients presented with partial proximal tubular dysfunction (partial Fanconi syndrome) that resolved after alkali treatment. However, the underlying mechanisms are unclear and have been discussed to be associated with the role of the proton pump in receptor-mediated endocytosis, and the co-expression of the a4 subunit together with the chloride transporter CLC-5 in the proximal tubule cells and α-intercalated cells of the collecting ducts [68]. As described by Palazzo et al., nephrocalcinosis was very common and detected in all but one patient and reported to present with different degrees (mild to moderate or marked). In addition, 3 patients developed kidney stones while there was no correlation between the severity of nephrocalcinosis and development of kidney stones. Also in this cohort a significant

number of the children presented with CKD (KDIGO G2, eGFR of 60-90 ml/min/1.73m^2) at last follow-up (at the age of up to 15 years). There was no significant correlation between the genetic diagnosis and CKD, however, there was a trend towards ATP6V0A4 mutations being more common in patients with CKD at last follow-up. This observation is supported by animal data from Atp6v0a4-deficient mice that demonstrated impaired proximal tubule function [42]. Furthermore, in this study data analysis from a total of 99 patients with *ATP6V1B1* and *ATP6V0A4* mutations demonstrated a more severe phenotype in patients with *ATP6V0A4* mutations compared to patients carrying the *ATP6V1B1* mutation.

In summary, clinical outcome of inherited dRTA patients seems to be good, particularly if diagnosis was established early with subsequent initiation of alkali treatment. In contrast to frequent presence of nephrocalcinosis and kidney stones in this population, some patients may develop CKD. The underlying mechanisms of CKD have not been fully identified yet and seem to be associated with the respective gene. Further studies are required with larger patient cohorts and longer follow-ups, especially from the time period after transition to adult care, to evaluate the risk to progress to end-stage renal disease.

Pregnancy

Female CKD patients are at increased risk for complications during pregnancy and therefore intensive monitoring and interdisciplinary care is highly recommended in this population [69-71]. However, patients with inherited dRTA usually have a normal kidney function with preserved estimated GFR (eGFR) and therefore are often not perceived as CKD patients. Nevertheless, several case reports have described severe complications during pregnancy in female patients with different types of RTA [72-75]. We have recently reported a series of three pregnant women with inherited dRTA with exacerbated acid-base disturbances during pregnancy [76]. All three patients presented with profound hypokalemia and worsening of metabolic acidosis during pregnancy. In addition to a potentially higher requirement for alkali therapy and potassium supplementation during pregnancy, physicians have to pay particular attention to hyperemesis gravidarum that might be a cause of stopping intake of alkali therapy and subsequent deterioration of acid- base and electrolyte

status. Other complications such as recurrent urinary tract infections and obstruction should also be considered because of pre-existing nephrocalcinosis and/or kidney stones. Consequently, in pregnant women with inherited dRTA interdisciplinary management including the obstetrician and the nephrologist is recommended. Furthermore, in addition to regular monitoring of creatinine and proteinuria, acid-base and electrolyte status should also be tested regularly to prevent life-threatening hypokalemia and decompensation of metabolic acidosis.

Stone risk in heterozygous carriers?

A recent study from Dhayat et al. has investigated the *in vivo* impact of a single-nucleotide polymorphism (SNP) in the coding region of the B1 subunit causing a change in amino acid sequence (c.481G.A; p.E161K) of the H^+-ATPase that causes greatly diminished pump function *in vitro,* and on urinary acidification in recurrent kidney stone formers [58]. Among 555 patients with stone disease, 5.8% were heterozygous for the respective SNP and demonstrated a trend to higher urinary pH values. 52.4% of the patients with p.E161K SNP were even identified with incomplete dRTA by using the short NH_4Cl loading test to confirm a urinary acidification defect in these patients (= urine pH > 5.3). As expected, there was a higher prevalence of calcium phosphate stones in p.E161K carriers when compared to wild-type subjects. As mentioned above, the simultaneous furosemide and fludrocortisone test is a valid alternative to the ammonium chloride test. Thus, Shavit et al. compared the results of both f+f and short NH_4Cl test from recurrent stone formers who were screened for dRTA [57]. Urinary acidification defect as a result of incomplete or complete dRTA was present in 50% of the 34 patients that were subjected to both tests. The comparison of both tests revealed a sensitivity of 100% but a specificity of only 24% for the f+f test. Therefore in patients with abnormal f+f test who are clinically not suspicious of defective urinary acidification, confirmation by NH_4Cl test should be performed.

THERAPY OF INHERITED DRTA

To date, therapy of inherited dRTA consists of alkali treatment to correct metabolic acidosis and avoid complications such as failure to thrive, growth

retardation, and rickets [46]. Physicians have to consider that in contrast to adults that usually require stable and low doses of bicarbonate such as 0.5-1 mEq/kg/day, growing children and infants may need substantially higher doses, especially if genetic diagnosis includes mutations in the B1 or a4-subunit of the H^+-ATPase compared to patients with *SLC4A1* mutations. Unfortunately, there is no amelioration of the progressive hearing loss and progressive nephrocalcinosis by alkali therapy. Potassium containing formulations such as potassium citrate should be preferred since patients usually present with a hypokalemic metabolic acidosis. However, potassium citrate may be unpleasant for some patients because of gastrointestinal side effects, therefore also sodium bicarbonate or other alkali formulations can be used or added to therapy. Pediatricians may also use Shohl's solution containing sodium citrate that can be easier dose-adjusted in children. In the presence of severe hypercalciuria thiazides can be administered to reduce renal calcium excretion, however, they should be used carefully since the risk of hypokalemia and polyuria may increase. If polyuria is severe indomethacin can be added to therapy.

Because of progressive and irreversible hearing loss hearing devices and language teaching are inevitable and thus of prime importance to ensure normal intellectual development and social integration of these patients.

SUMMARY AND CONCLUSION

dRTA is a rare inherited tubular disorder impairing the kidneys ability to acidify urine and excrete acid. The clinical manifestations depend on the gene mutated. In severe cases patients may present after birth with failure to thrive, vomiting, dehydration, and profound disturbances of acid-base balance and electrolytes. In milder cases, nephrocalcinosis or -lithiasis may be the first clinical presentations. Next to treatment of metabolic acidosis, the progressive loss of hearing should be treated with hearing aids to ensure a normal intellectual development of children. Early genetic diagnosis and counseling of parents is important. During pregnancy, women with dRTA may suffer from exacerbations of their metabolic acidosis and experience severe electrolyte disturbances requiring a close monitoring of these parameters.

Figure Legends

Figure 1. Scheme of mechanisms underlying bicarbonate reabsorption in the proximal tubule. *NBCe1* electrogenic Sodium-bicarbonate-cotransporter 1, *NBCn2* electroneutral Sodium-Bicarbonate-cotransporter 2, *NHE3* Sodium-Proton-exchanger 3, *V-ATPase* vacuolar-type H^+-ATPase, *CAII* Carbonic anhydrase type II, *CAIV* Carbonic anhydrase type IV.

Figure 2. Type-A acid-secretory intercalated cells in the collecting system and structure of the V-type H^+-ATPase (insert). The red/orange parts of the pump belong to the V_1-partl, the blue subunits to the V_0-sector; the B- and a-subunits, mutated in inherited dRTA, are indicated and occur in different isoforms. The B1 or a4 isoforms, respectively, can be mutated in patients with dRTA and nephrocalcinosis. *AE1* „anion exchanger“ 1, *RhCG* „rhesus blood group family type C glycoprotein“, *RhBG* „rhesus blood group family type B glycoprotein“, *CAII* Carbonic anhydrase type II, *V-ATPase* vacuolar-type H^+-ATPase)

Figure 3. Medullary nephrocalcinosis as a typical feature of patients with inherited distal RTA: CT scan (left and center panel), plain abdominal radiography (right panel)

Figure 4: Enlarged vestibular aqueduct in a patient with inherited dRTA:
MRI images of the temporal bone and labyrinth with bilateral enlarged endolymphatic duct (arrow) (A). (B) Three-dimensional reconstruction of the labyrinth showing the enlarged endolymphatic duct and sac (arrow) and bulbous dysplasia of the apical turn of the cochlea (short arrow). Right (**c**) and left (**d**) temporal bone with enlargement of the bony vestibular aqueducts (long arrow) in comparison to the diameter of the posterior semicircular canal (short arrow). Taken from [36].

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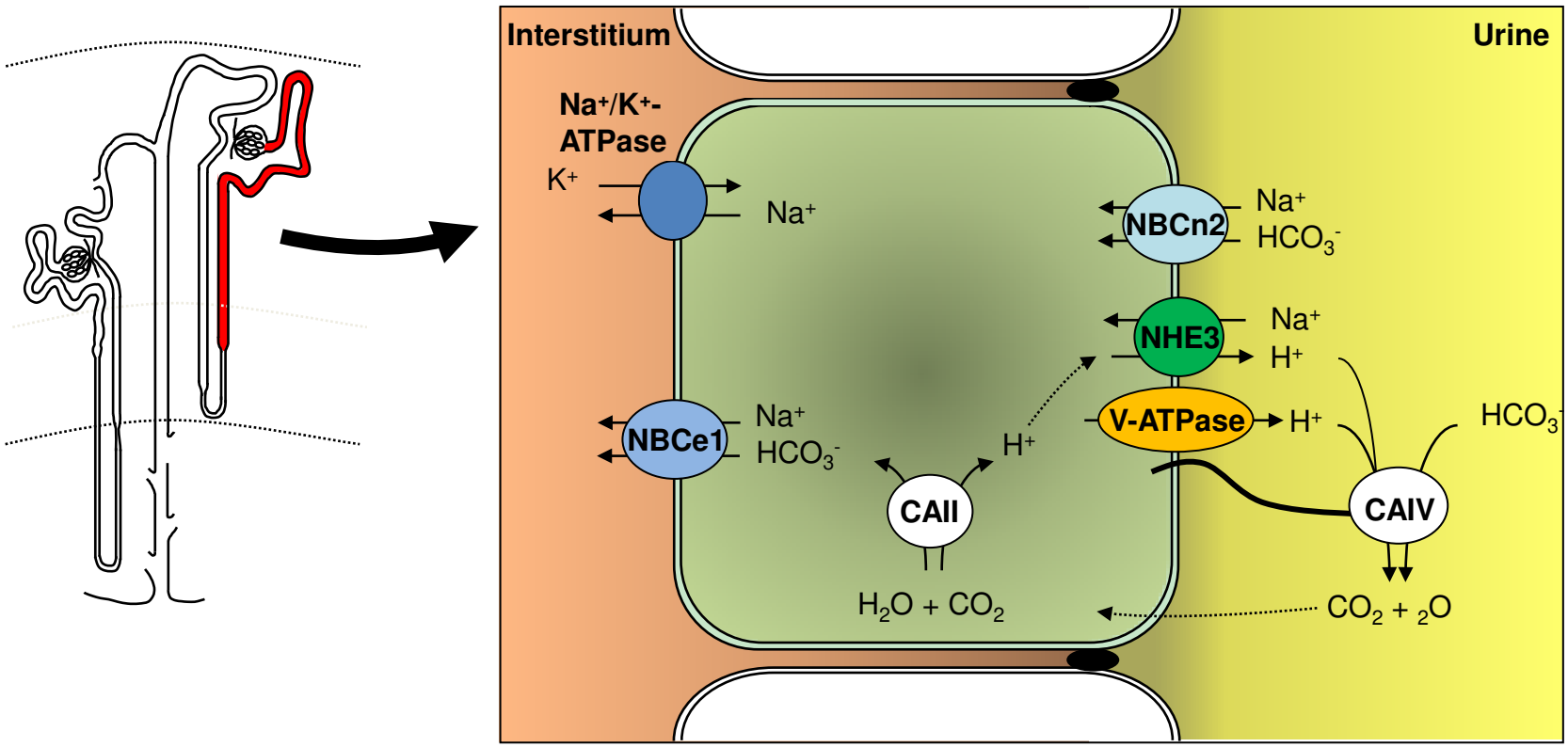
Figure 1


Interstitium
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ATPase
K+
Na+
NBCn2
Na+
HCO3-
NHE3
Na+
H+
NBCe1
Na+
HCO3-
V-ATPase
H+
HCO3-
H+
CAII
CAIV
H2O + CO2
CO2 + 2O

Figure 2

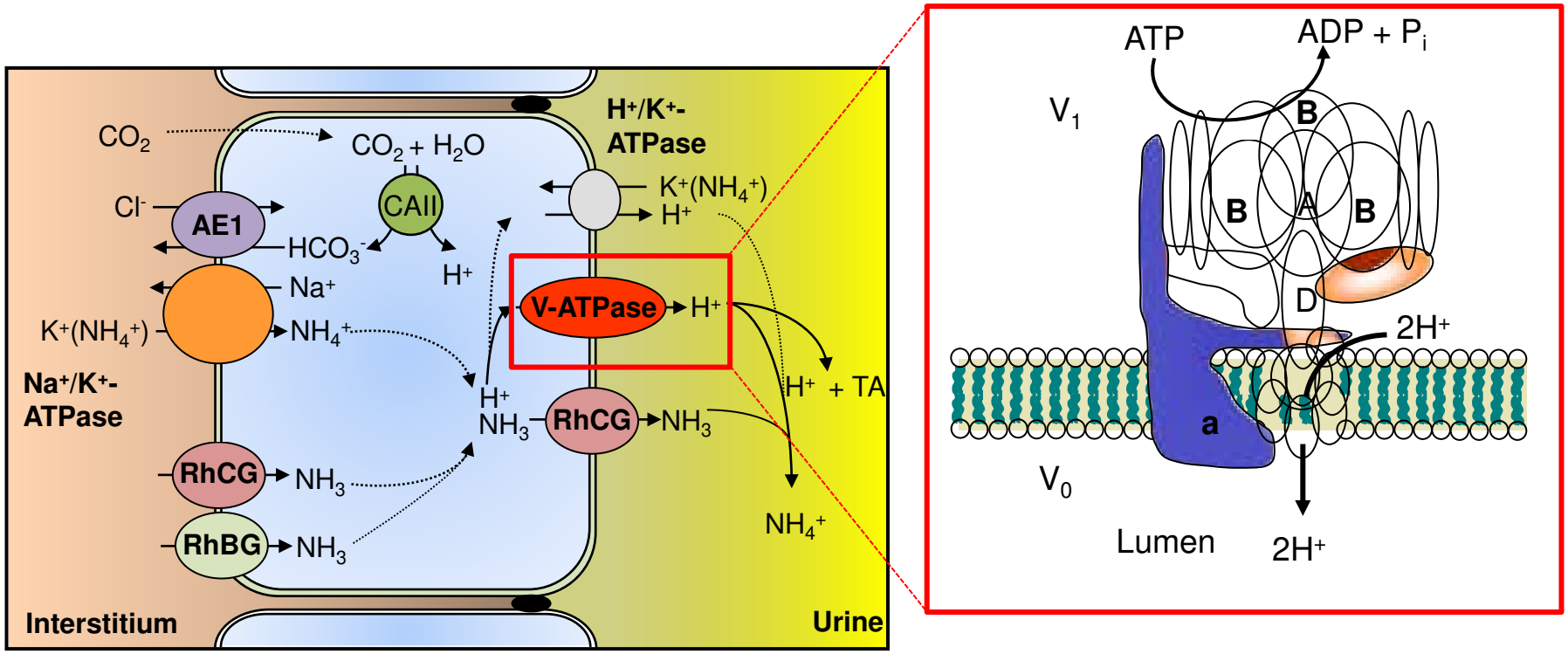
CO2
CO2 + H2O
Cl-
AE1
CAII
HCO3-
H+
Na+
K+(NH4+)
NH4+
Na+/K+-
ATPase
H+/K+-
ATPase
K+(NH4+)
H+
V-ATPase
H+
H+ + TA
H+
NH3
RhCG
NH3
NH4+
RhCG
NH3
RhBG
NH3
Interstitium
Urine
ATP
ADP + Pi
V1
B
B
A
B
D
2H+
a
V0
Lumen
2H+

Figure 3

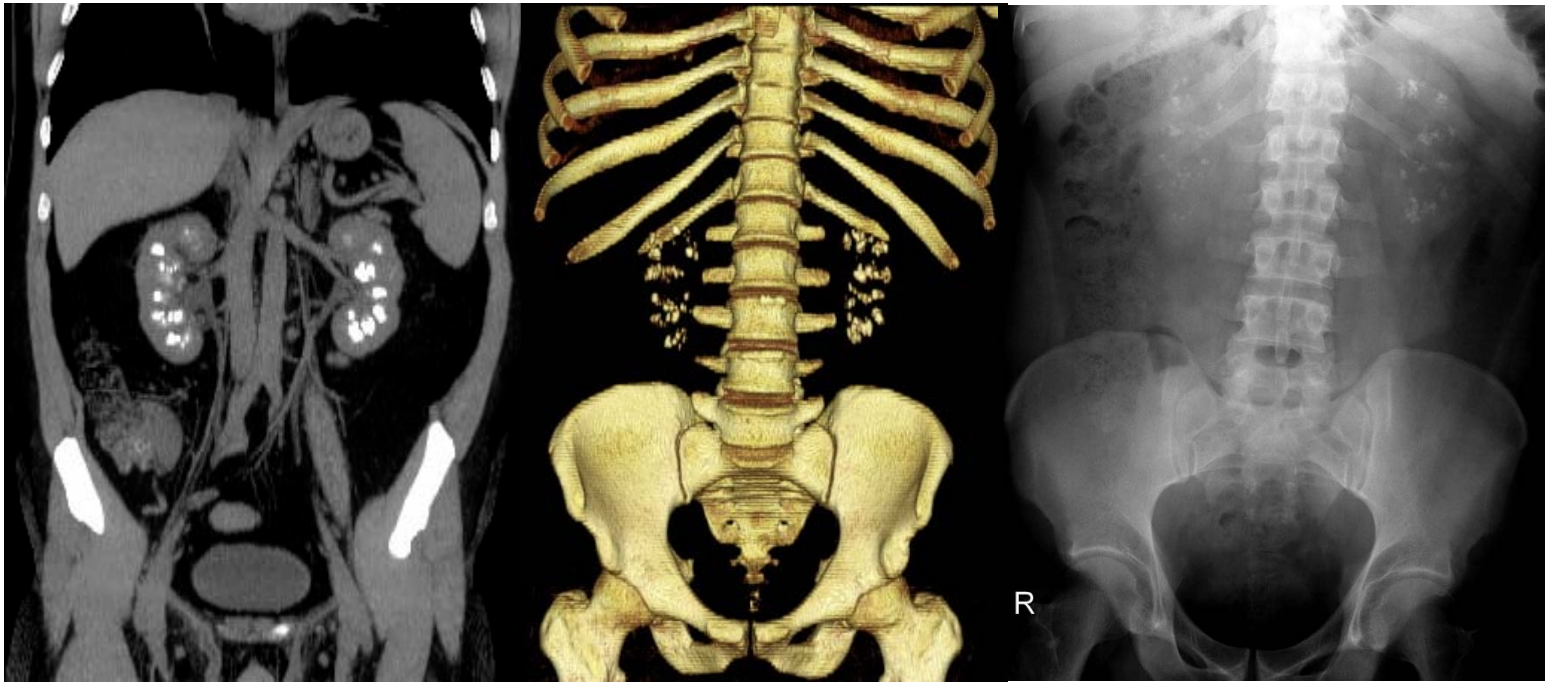

Figure 4